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The Action of Various Pharmacological and other Chemical Agents on the Chromatophores of the Brook Trout Salvelinus Fontinalis Mitchill

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From the Department of Zoölogy, University of Wisconsin

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The reactions of melanophores (pigment cells) to pharmacologically active agents have been but little investigated. In the

majority of the physiological researches upon the melanophores, the experiments have only included the study of such physical agents as light, heat, etc. The problem here undertaken was to determine the reactions of the melanophores of young trout embryos in response to changes in their chemical environment. The trout embryos that were used in these experiments were too young to react to a change in the light conditions, and throughout the work gave no evidence of any psychic influence of the pigment cells.

MATERIAL AND METHODS

Young brook trout embryos, from two days to two weeks after hatching, were used. The melanophore of such young individuals are dark, much branched cells with deep black or brown pigment granules. These are the only kind of pigment cells present at this time. The xanthophores, the yellow or reddish pigment cells, appear after or a little before the yolk is absorbed. All the experiments were performed before the xanthophores appeared. After the yolk is absorbed the fish begin to react to the back ground. When placed in a dark dish, they become dark; when placed in a white dish, light in color. Microscopical examination shows that the pigment cells (melanophores) are expanded in the dark colored individuals and contracted in the light ones. The very young, two-day or two-week old embryos do not respond to changes of the back ground.

This constant condition is taken as a known factor. The contraction of the pigment cells was used as the criterion for determining stimulation, and their expansion (relaxation) as a mark of depression. The expansion of the pigment cells is characterized by the peripheral migration of the pigment granules within the processes of cell, and in contraction the movement is centripetal. My reason for considering contraction as stimulation and expansion as a depression is that certain reagents, alkaloids for example, if used in high concentrations produce no observable change in the pigment cells which under normal conditions are expanded. Small or 'therapeutic' doses produced a

contraction. Large doses produced an expansion of all the cells which had contracted in the weak solution. Inasmuch as it has been shown by various investigators that large doses of pharmacologically active agents produce a depression, and small doses incite a stimulation in other tissues, it is inferred that the condition is essentially the same with the melanophores.

All the chemicals used in these experiments were of Merck's, Kahlbaum's and Baker's manufacture. The solutions were made up with oxygenated distilled water. Chemically pure oxygen was bubbled through the water before it was used. This precaution was taken because the distilled water was very low in oxygen content and in it the pigment cells contracted. When oxygen was added no such contraction occurred. The details of the way in which the solutions were prepared are given under the respective heads.

The experiments with the salts and the alkaloids were carried on in Syracuse watch glasses, which were kept covered to prevent excessive evaporation. They were uncovered only when actual observations were made. The amount of the solution used was about 10 cc. Experiments were performed in stender dishes of 50 cc. capacity as a check on the Syracuse watch glasses. There was no difference in the results. The experiments with volatile substances were carried on in wide-mouthed, glass stoppered bottles, with a capacity of 50 cc. All precautions were taken to prevent evaporation.

Most of the experiments were carried on at room temperatures which varied between 69° and 72° F., although some were performed at the fish hatchery where the temperatures were from 46° to 50° F.

The experiments with the alkaloids and alcohols were started in solutions of 0.0001 per cent. The concentrations were increased in multiples of ten.

The experiments were repeated ten to fifteen times for each solution tested. In many cases the experiments were repeated double the number, in order to eliminate all possible individual variation and errors.

I wish, here, to express my chief indebtedness to Prof. M. F. Guyer, for his kindly criticism and suggestions during the progress of the work. To Prof. A. S. Loevenhart, I wish to acknowledge my appreciation of many courtesies extended. For the privilege and use of the fish hatchery and trout embryos, I desire to express my appreciation of the favor to Dean E. A. Birge and Superintendent James Nevin of the Wisconsin Fish Commission.

Reactions to gases

1. Oxygen. The oxygen used in these experiments was chemically pure. The pigment cells remained expanded in an atmosphere of oxygen, and the fish lived indefinitely.

The hydrogen used in these experiments was obtained by the action of chemically pure hydrochloric acid on Merck's highest purity zinc. The gas was passed through two towers of KOH and then through two towers of distilled water, of which one had red litmus, and the other blue litmus. The trout were in the fifth tower.

The pigment cells contracted completely in four to six minutes when the embryos were exposed to hydrogen. If oxygen was substituted before the fish died the pigment cells expanded. If the oxygen was again replaced by hydrogen the pigment cells contracted. The results of these experiments show (1) that the absence of oxygen caused a contraction of the melanophores; (2) that the oxygen is necessary for the maintenance of the expanded pigment cells.

2. Carbon dioxide. The carbon dioxide was generated through the interaction of chemically pure hydrochloric acid on marble. The gas was purified by being passed through a tower of sodium bicarbonate and then through a tower of acidified lead acetate, and lastly through two towers of distilled water.

The fish were exposed to water through which the carbon dioxide was bubbling in a steady slow stream. The carbon dioxide produced a complete contraction of the pigment in two and one-half minutes. The time of contraction was the same for all the

experiments performed. If an intense stream of oxygen was bubbled at the same time with the carbon dioxide, the pigment cells remained expanded. The proportion of the two gases which maintained the expansion of the melanophores was not determined. Briefly summarized the results prove that carbon dioxide produces a contraction of the pigment cells of trout embryos. The presence of oxygen antagonized the action of the carbon dioxide.

Effects of distilled water

The first experiments that were performed were to determine the effect of distilled water on the pigment cells of trout embryos. The normally expanded pigment cells contracted in ten to twelve minutes and the fish died usually in about twenty minutes—differing somewhat with the individual lots of fish. After an interval of ten to thirty minutes, following the initial contraction, the pigment cells began to expand. This secondary expansion of the melanophores in no way equaled the normal expanded condition. The processes of the cells were short and blunt. This expanded condition lasted for a short period; then the walls of the melanophores began to break down and the cell contents, viz., the pigment granules migrated into the interspaces of the epidermal layer. Often the pigment cells disintegrated without a previous expansion. Spaeth ('13) obtained essentially the same results with isolated scales of Fundulus in which the chromatophores (1) expanded, (2) contracted, (3) expanded a second time with a final degeneration. He did not try oxygenated distilled water. If 2 cc. of boiled tap water were added to 8 cc. of distilled water the results were the same. Then boiled tap water was tried and the pigment cells contracted in fourteen and twenty-two minutes. In distilled and boiled tap water through which oxygen had been bubbled the melanophores remained expanded and the fish lived indefinitely. The conclusion was obvious. It was oxygen want and not the absence of salts in the distilled water that caused the contraction of the pigment cells and the death of the fish.

Reactions to salts

The problem of salt action is one of the most interesting within the scope of physiology and has wide applications. The relation of various salts to heart beat is a long debated question.

Howell ('98), p. 49, is of the opinion "that the inorganic salts of the blood and liquids of the heart tissues especially of the calcium compounds, stand in a peculiar and fundamental relation to the initiation of the inner stimulus of the heart contractions." Loeb ('00 a, '00 b) believes that the sodium cations acting on the striped muscle to be the stimulating agents being counteracted by the ions of potassium and calcium. The position of Loeb is supported by Lingle ('00). Benedict ('05 and '08) is of the opinion that the anion probably plays an important rôle in the action of salt solutions upon heart beat.

Mathews ('04 a, '04 b, '05, '06) maintains that in the action of salt solutions on motor nerves, colloids, and sea urchin eggs, the ionic potential of the salt, which is the reciprocal of the solution tension, is an important factor in ionic action. R. S. Lillie ('11, '12 a, '12 b) working with the larvae of Arenicola and eggs of starfish, and McClendon ('10) on sea urchin eggs put forth the hypothesis that ionic action is due to the modification of the permeability of the plasma membrane. Loeb ('00 b) holds that ionic action is due to the formation of ion protein compounds, that is that the ions of the salt combine directly in some way with the protein molecules of the living protoplasm. True and Kahlenberg ('96) working with plants (Lupinus albus) believe that the anion is unimportant in the toxic action of the salt.

Spaeth ('13) working on the chromatophores in isolated scales of Fundulus heteroclitus concludes that the anion in potassium salts is of no importance in causing the initial contraction of the chromatophores, but that in the secondary expansion of the chromatophores the action of potassium is modified by the anions. On the other hand, the duration of the sodium expansion varies with the nature of the anion.

The above opinions tend to show that the part played by ions in stimulation is by no means a settled question. In an attempt to gain further insight into the subject brook trout embryos were subjected to solutions of pure potassium and sodium salts. The results have been so promising that the work is being extended to numerous other salts.

The salts used were of the purest of Merck's, Kahlbaum's and Baker's manufacture. The solutions were made up in a 0.2 molecular concentration with oxygenated distilled water. The solutions of the iodides which readily undergo decomposition were never older than thirty-six hours when used.

The experiments were carried on in Syracuse watch glasses in about 10 cc. of the solution. At times small dishes of 25 to 50 cc. capacity were used.

1. Effects of potassium salts. When the trout embryos are immersed in a 0.2 M. KI solution a rapid contraction of the normally expanded chromatophores results within two or three minutes. They then appear as minute dots with no peripheral processes. In placing a similar lot into a 0.2 M K_2SO_4 equivalent solution the change does not occur as rapidly, being completed in fifteen to twenty minutes. This at once suggested that there is a specific difference in the rate of contraction for potassium, varying with the anion. The experiments were extended to include the following neutral salts of potassium, viz., K_2SO_4, KCl, KBr, KNO_3 and KI. Practically the first experiment showed that there was a distinct difference in the rate of contraction varying with the anion. The rate and intensity of the contraction was most rapid in the order given (figs. 1, 2, 3, 4 and 5).

$$I > \quad NO_3 > \quad Br > \quad Cl > \quad SO_4 >$$

In KI the contraction was complete before it had even begun in KCl or K_2SO_4. The experiments were repeated many times and as a check several of my colleagues were asked to come in and arrange the sets showing the greatest change. In all cases their arrangement was in the above order. This clearly indicates that if contraction in the melanophore is specifically induced by the

cation of potassium, it is unqualifyingly modified by its anion or the residual part of the undissociated molecules.

Another interesting feature observed was that after a longer or a shorter interval after the first contraction there followed a peripheral expansion of the pigment cells (figs. 6, 7, 8, 9 and 10), that is, the pigment cells put out processes which became longer and longer as time went on but which never reached the original size they had before treatment with the potassium salt solutions. This expansion set in earlier in KI where the contraction took place first, evidently the secondary expansion or paralysis is reciprocal of the first contraction. The expansion is in the order of the first contraction (figs. 6, 7, 8, 9 and 10).

$$I > \quad NO_3 > \quad Br > \quad Cl > \quad SO_4$$

This peripheral migration of the pigment is in the nature of a paralysis. The paralytic state (depression) is soon followed by death of the pigment cell. The walls of the pigment cell disintegrate and the pigment granules flow into the interspaces of the body tissues. Death of the cells takes place often before the expansion is complete, and then premature disintegration of the pigment cells occurs. The condition or extent of the degeneration is dependent upon the 'physiological state' of the melanophores and the individual fish.

The maintenance of the irritability of the melanophores followed the same order, correlated with this was the longevity of the fish. The fish lived the longest in K_2SO_4 and KCl. They died very rapidly in KI.

The reactions varied with the concentration of the solutions, for in solutions of 0.1 M or less the changes were slightly slower. Molecular solutions gave no results but killed the fish immediately.

2. Effects of sodium salts. Here as in the potassium salts the embryos used had their melanophores expanded. It was observed that the neutral salts of sodium produced a contraction of the melanophores very slowly. In some instances the contraction did not take place in 92 to 116 hours, especially in the solutions of Na_2SO_4 and NaCl. The contraction in NaI was complete

in five to forty-five minutes. It was confirmed by repeated observation, that these contractions, slow as they may be for certain solutions (Na_2SO_4 and NaCl), were in the following order:

$$I > \quad NO_3 > \quad Br > \quad Cl > \quad SO_4$$

A number of experiments were tried to determine if the sodium salts produced an expansion of the melanophores after the potassium salt contraction. The embryos were exposed to KCl from fifteen to twenty minutes when they were removed and rinsed in water to free them of the excess of KCl. They were now placed into the five neutral salts of sodium. The rate and degree of expansion was in the following order:

$$SO_4 > \quad Cl > \quad Br > \quad NO_3 > \quad I$$

The expansion was most rapid and complete in Na_2SO_4 and NaCl. In NaI there was no expansion.

The experiments were repeated with embryos that were not rinsed with water. The result was the same as in those that were washed in water. If the melanophores are contracted with KI instead of KCl the results are the same.

$$SO_4 > \quad Cl > \quad Br > \quad NO_3 > \quad I$$

It is interesting to note here that no expansion of the melanophores occurred in the NaI solution. Is this because the sodium cations are inhibited in permeating the cell membrane due to the presence of the dissociated iodine anions or some other factor? Are the cells permeable only to the iodine anions and not to the cations of sodium? Hamburger and von Lier ('02) claim that the blood corpuscles are permeable only for anions and are not permeable to the cations. If the expansion of the melanophore is specific for the sodium cation, it is overcome by the antagonistic action of the iodine anion, which produces a contraction. Nevertheless we must consider another factor, that is, the action exerted by the residual undissociated molecule which is present at all times in the solution. The expansion in-

duced by the sodium salts after a potassium salt contraction is followed by a contraction of the melanophores in the usual order. The position or order of the contraction was the same as for the expansion of the melanophores; but with one exception where the $NaNO_3$ changed places with the NaBr.

$$SO_4 > \quad Cl > \quad NO_3 > \quad B_r > \quad I$$

The extent to which the life of the fish and the irritability of the melanophores are preserved is possibly the function of the cation which is modified by the anion or the residual undissociated molecule.

3. Discussion. All these results seem to lend themselves to the interpretation that salt solution having a common cation are modified by their anions or the residual undissociated molecule. This is clearly shown by the rate and degree of the contraction of the melanophores by the potassium salts, where the contraction may be specific for the cation of potassium. Speath ('13) p. 547 says in speaking of the action of potassium salts: "The time of this contraction (K) is the same for the five salts within the limits of the variation of the individual scales. Since there is this common cation K^+ in all five salts it seems probable that the initial effect (contraction) is specific for the K^+ ions." My own results in the case of pigment cells of trout embryos are contrary to this conclusion. If contraction is specific for the positive cation of potassium (K^+), it should be the same in rate and degree in all the salts of potassium. Since the rate and degree of the contraction are not the same for the five potassium salts (figs. 1, 2, 3, 4, and 5) it must depend on some other or some modifying factor which is responsible for this difference.

A dissolved electrolyte conducts a current in proportion to the extent that it is dissociated or ionized. Its maximum conduction will be at complete ionization which occurs at infinite dilution. Therefore the degree of the dissociation or the coefficient of dissociations can be obtained from the conductivity of solution. The conductivity of an electrolyte divided by its num-

ber of gram equivalents in cms. is the molecular conductivity of the substance written as Λ. However, the conductivity is at its maximum at infinitely dilute solutions, therefore the value $\Lambda\infty$ is taken as a measure of the total number of ions that are produced by the dissociation of one gram equivalent of the substance. Therefore d the degree of dissociation is directly proportional to the conductivity; thus we have the simple formula $\alpha = \frac{\Lambda}{\Lambda\ \infty}$. The equivalent conductivity at infinite dilution for KCl is calculated to be 130.10. The equivalent conductivity of a two-tenth molecular KCl is 107.96 $\Lambda_{0.2\ M}$. The degree of dissociation at 18°C. is the ratio $\frac{\Lambda\ 0.2\ M}{\Lambda\ \infty}$, or $\frac{107.96}{130.10}$ or 82.98 per cent. The values obtained in this way may be regarded only as approximate. The values are given in the following table.

TABLE 1 *

SALT	$\frac{1}{2}K_2SO_4$	KCl	KBr	KNO_3	KI
Equivalent conductivity at infinite dilution $\Lambda\ \infty$	132 8	130 10	132 30	126 50	131 10
Equivalent conductivity at 0.2 M dilution Λ 0.2 M	87.76	107.96	110.40	98.74	111.2
Per cent or degree of dissociation $\alpha = \frac{\Lambda\ 0.2\ M}{\Lambda\ \infty}$	66.03	82.98	83.44	78.05	84.82

A study of the table leads one to believe that the rate and the degree of the contraction are in some way correlated with the degree of dissociation of the salts. The lowest rate and degree of contraction was found in K_2SO_4, where the degree of dissociation is 66.03 per cent. The most rapid and complete contraction occurred in KI where the dissociation is 84.82 per cent.

Potassium nitrate is out of place. It has a greater stimulating action than its degree of dissociation would indicate. It should fall between potassium sulphate and potassium chloride. The possible explanation for this break in the series may be that the

TABLE 2

SALT	½ Na_2SO_4	NaCl	NaBr	$NaNO_3$	NaI
Equivalent conductivity at infinite dilution $\Lambda \infty$	111 5	108 99	112.0	105.99	109.9
Equivalent conductivity at 0.2 M dilution Λ 0.2 M	71.4	87.73	91.2	82.28	90.2
Per cent or degree of dissociation $\alpha = \frac{\Lambda\ 0.2\ M}{\Lambda\ \infty}$	64.03	80.49	81.43	78.11	82.08

nitrate anion exerts an independent action or it may form nitrites which are more active.

In table 2 are shown the equivalent conductivities and degree of dissociation of the sodium salts.

The values were calculated in the same manner as those for the potassium salts. Here, as in the potassium salts, the reaction of the melanophores was correlated with the degree of dissociation.

There are two reactions of the melanophores which are characteristic of the potassium salts: (1) a primary contraction, (2) an expansion which is the sign of death or degeneration of the cell. The cell wall breaks down and the pigment granules escape into the surrounding tissues. The degree of the cytolysis is directly proportional to the degree of dissociation of the salt. In sodium salts we have two specific reactions: (1) the expansion and maintenance of the expansion for a certain period of time, (2) a slow contraction. The two reactions of sodium salts occur in an inverse order to those of the potassium salts, where contraction is followed by a cytolytic expansion. The contraction in sodium salts is not followed by a cytolytic expansion, but the disintegration takes place directly from the contracted pigment cell. This contraction in sodium salts is directly comparable to the cytolytic expansion observed in potassium salts, for both of these stages indicates the death of the pigment cell.

A. P. Mathews ('06) suggested that it is the ionic potential of the ions, and not the difference of voltage between the plate of

a metal and any solution of its salts, but rather the difference in pressure between a single ion and a single atom of the metal that determines the chemical action of the ions. Since solution tension is a measure of the difference in potential between the solution which contains a known amount of the ions of the metal and the metal itself, it is also the difference between the tendency of an atom of the plate to become an ion. When applied to living protoplasm the metal plate is replaced by the protoplasm. The value varies with the amount of electrolytic dissociation and the kind of plate present.

The solution tensions in volts of elements in normal ionic solutions.

		Cl	1.694
K	2.92	Br	1.270
Na	2.54	I	0.797
		NO_3	2.229

The ionic potential is the reciprocal of the solution tension. Ionic potential is the tendency of any ion in any concentration of solution to change into an atom of its metal.

The ionic potentials of the ions of metals in volts are:

		Cl	1.694 (?)
K	2.92 (?)	Br	1.270 (?)
Na	2.54 (?)	I	0.797 (?)
		NO_3	2.229 (?)

Mathews ('06) shows that the dissolving power of the salts of sodium and potassiun for edestine, a globulin of the hemp seed is in some way correlated with the ionic potential.

SALT	IONIC POTENTIAL	NUMBER OF CUBIC CENTIMETERS REQUIRED TO DISSOLVE ONE GRAM OF EDESTIN
KI	−2.123	5.7
KBr	−1.65	10.0
KCl	−1.226	15.1
NaI	1.743	5.7
NaBr	−1.270	9.3
NaCl	−0.846	12.8

The more negative the value for the ionic potential the greater the solvent power of the salt for edestin. The negative value in potassium is much greater than that in the sodium. In the table we observe that it takes like amounts of the iodides and less of the other sodium solutions to dissolve the edestin. However, we should expect it to take less of the potassium salts than it does of the sodium. I find this to be true for the pigment cells of trout, where the potassium salts cause the contraction of the pigment cells more rapidly than do the salts of sodium. Unfortunately the solutions tensions for sodium and potassium are more or less indefinite which makes the results obtained for the salts of these metals incomparable. The ionic potential is not determined directly, but calculated only, thus making the explanation more difficult.

The results obtained in experiments on the action of salts on the pigment cells of trout are explicable on three assumptions; (1) that it is the antagonistic action between anion and cation, (2) that it is the independent action of the cation, (3) that the reaction is modified by the residual undissociated molecule.

The antagonistic action between anions and cations has been postulated by Mathews ('06), Benedict ('05, '08), and W. Koch ('09). The increased action of different salts having the same cation have been observed in different tissues. Loeb ('99) produced a better rhythmical contraction in striped muscle with NaI than he did with NaCl. Zoethout ('04) confirmed this observation, and extended it to KI which increased the muscle tone more than KCl. Benedict ('08) concluded that "the direct production of rhythmic activity by means of a salt's action upon heart muscle is due to the anion of the salt, while the chief function of the cation is apparently to maintain such a tone of the heart muscle that it will respond to the stimulus furnished by the anion." Mathews ('02) has shown that the presence of iodine, bromine anions stimulated the motor nerve more powerfully than the chlorine anion. Speath ('13) observed that the cytolytic expansion of the melanophores in potassium solutions, varied with the anions, but he did not note a difference in the rate of the primary contraction of the melanophores in the

different potassium salts. In sodium salts the expansion of contracted melanophores varied with the anion, and the contraction following this expansion was correlated with the anions.

In neutral salts of potassium there are two constant results produced on the pigment cells of trout; (1) a contraction of the pigment cells, (2) a cytolytic expansion. The times for each varied with the anion. If the antagonism existed between the potassium cations K +, and the negative anions SO_4-, Br−, Cl−, No_3-, I−, it was the least effective in KI and most potent in K_2SO_4. The order of contraction and expansion was

$$I > \quad NO_3 > \quad Br > \quad Cl > \quad SO_4$$

In sodium salts there were two characteristic reactions, (1) an expansion, (2) a contraction. The rate and degree of the expansion of the melanophores was greatest in Na_2SO_4 and least in NaI. The rate of contraction was rapid in NaI and least in Na_2SO_4. The order of the expansion was

$$SO_4 > \quad Cl > \quad Br > \quad NO_3 > \quad I$$

The contraction rate of the pigment cells was inverse to the above.

$$I > \quad Br > \quad NO_3 > \quad Cl > \quad SO$$

The cationic action was modified by the nature of the anion. This anionic order was observed by Paul and Kronig ('96) on the disinfecting power of mercuric salts of chloride bromide and cyanide. Mathews ('06) has shown for the eggs of Fundulus heteroclitus that the fatal dose varied with the anion. Loeb and Cattell ('15) have shown that the hearts of Fundulus embryos, previously poisoned by KCl, and recovered by sodium salts was an anion effect inasmuch as it increased with the anion, apparently in agreement with Hardy's rule (ion effect = exponential function of the valency) for the acetate was much more efficient than the chloride.

2. That it is the cation of potassium or of sodium that causes the reaction of the pigment cells of trout embryos.

Loeb ('10, '12) and Loeb and Wasteneys ('11 a and '11 b) maintain that there is an antagonism between the sodium cation

Na + and the potassium cation K + and not between the potassium cation and the chlorine anion K + Cl −. This is supported in part by the foregoing experiments on the pigment cells of trout embryos. The pigment cells are expanded in sodium salts after a potassium salt contraction. But this is not true of all the salts of sodium. If the pigment cells are contracted in KCl or KI and are now placed in NaI there is no expansion. Apparently there is an antagonism between the dissociated anions of (Cl − and I −) and the sodium cation (Na +) for from the conditions of the experiment we should get an expansion. It is probable that Loeb underestimated the antagonism between the positive ions of K + and Na + and their negative ions Cl −. The longevity of the fish is better protected in sodium salts than in potassium salts. But again some of the sodium salts are more protective (Na_2SO_4 or NaCl) than others (NaI). That the potassium and sodium cations do exert some such modifying action is undeniable, but to say that it is independent of its anion is not warranted by the facts at our command.

3) That it is the residual undissociated molecules in the solution that modify the action of the salt.

In 0.2 M, KI the degree of dissociation is much greater than in an equivalent 0.2 M, solution of K_2SO_4. Correspondingly KI initiates more intense responsiveness of the pigment cells than does K_2SO_4. The rate and degree of the reactions of the pigment cells decline as the number of the undissociated molecules increases. In the potassium salts the primary contraction and the expansion vary with undissociated molecule, thus,

$$SO_4 > \quad Cl > \quad Br > \quad NO_3 \quad I$$

The degree of dissociation for 0.2 M solutions are

66.03 82.98 83.44 78.05 84.82

The fact that the nitrate is out of place was mentioned before. As already stated, this may be due to the independent activity of the nitrate, which may break down to form a nitrite.

In sodium salts the dissociation percentages are slightly less than in potassium salts. The rate of contraction is much slower.

The fish live longer in sodium solutions, and the pigment cells retained their irritability longer. The pigment cells expand if transferred after they are contracted in potassium salts. Is this reaction specific for the sodium ion or is it due to the increased number of undissociated molecules in sodium solution? It cannot be positively concluded whether this difference in residual molecules is enough to account for the difference in the rate of contraction of the melanophores in sodium and potassium salt solutions. The ascribing of the principles of salt action to the anion or cation without the consideration of the residual undissociated molecule is just as out of proportion in the field of physiology as to say that the undissociated alkaloids and other substances have no action.

Reactions to alcohols

Whether or not alcohols have a stimulating action is a much debated question. The Schmiedeberg school of pharmacologists maintains that alcohols produce no primary stimulation of the central nervous system. According to this view the giving alcohol to a mammal, if followed by an increased muscular activity, is said to be due to the depression of the cerebral centers, thus removing the restraint from the motor areas. Binz and his followers hold to the view that alcohol first stimulates and then depresses the nerve cells.

The literature on the pharmacological action of alcohol on the heart and other tissues is very extensive, but to my knowledge there are no records of any attempts to determine its action on the melanophores. Whatever action the alcohols exert on the melanophores will not settle the question whether alcohols stimulate or depress the nervous system, as the melanophores in the very nature of their origin and structure must be looked upon as specialized mesenchymal cells. While it is not at all improbable that the general facts observed with melanophores may be

[1] After these experiments were completed Spaeth 1916 published results, where he subjected isolated scales of Fundulus to vapors of alcohol, ether and chloroform and always obtained a contraction of the melanophores, and larger amounts of these vapors inhibited the contraction.

true also of other tissues, I refrain from applying the results to such an interpretation. The literature is used in a comparative way, but not in the sense that the results obtained with melanophores are directly comparable.

Ten per cent stock solutions of methyl (Sp. G. O. 796), ethyl (Sp. G. O. 796–800) propyl (Sp. G. O. 8066) alcohols of Merck's manufacture were made up with oxygenated distilled water. The dilutions were made from these stock solutions with oxygenated distilled water. The experiments were carried on in glass stoppered bottles of 75 cc. capacity. All work was done at room temperature of 18° to 20°C.

1. Methyl alcohol. Overton ('01) showed that methyl alcohol has a less powerful narcotic action on tadpoles than ethyl alcohol. Vernon ('11) confirmed that the same was true in the depressing action of methyl alcohol on the heart muscle of a turtle's heart.

Young brook trout embryos of the same age and condition were subjected to the action of the respective alcohols of the various concentrations. The contraction of the pigment cells was taken as the criterion of stimulation, the relaxation (expansion) as that of a depression.

Ethyl alcohol in solutions of 1.6 to 2.5 per cent produced a complete contraction of the pigment cells. Methyl alcohol of an equal concentration did not cause a contraction. In a 3.5 per cent solution there was a slight retraction of the pigment cells, but the contraction was not complete. A 4.5 per cent solution produced a complete contraction of the melanophores. Solutions of 5 per cent to 5.5 per cent produced a slight contraction of pigment cells. This partial contraction was followed by an immediate expansion. If embryos in which the melanophores were just contracted in a 0.005 per cent strychnine solution were subjected to 5 per cent to 5.5 per cent methyl alcohol the pigment cells expanded. In 7 per cent to 10 per cent solutions of methyl alcohol there was no visible change in the expanded melanophores.

Thus it may be concluded that (1) methyl alcohol in high concentration acts as a depressing agent, (2) in medium concentra-

tion it has a stimulating action, and (3) in very weak solution it has no effect on the melanophores of trout embryos. Methyl alcohol has a less pronounced stimulation action than ethyl alcohol on pigment cells of trout. It was necessary to double to concentration so as to bring about reactions in any way comparable to those produced by ethyl alcohol. The action of methyl alcohol was less striking and the stages of stimulation and relaxation were slower in appearing than in ethyl alcohol.

2. Ethyl alcohol.—When trout embryos were exposed to weak solutions (0.01 per cent to 0.8 per cent) of ethyl alcohol, no change took place in the pigment cells. The embryos did not show any signs of depression and appeared perfectly normal. In solutions of 1 per cent to 1.5 per cent the embryos became more restless and the pigment cells exhibited a partial contraction. In concentrations of 1.6 per cent to 2.5 per cent of ethyl alcohol the fish became more active, the pigment cells showed a complete contraction; while in solutions of 3.0 per cent to 4.5 per cent they showed a transitory contraction, followed by an expansion. This result could be very easily overlooked. In 6 per cent to 10 per cent solutions the trout embryos died rapidly in from fifteen to twenty-five minutes, and there was no contraction of the pigment cells. If embryos that had their pigment cells contracted in the 2 per cent solution were transferred to a 7 per cent the pigment cells expanded rapidly.

If the embryos in which the pigment cells were contracted were transferred to a 4.5 per cent to 6 per cent solution an immediate expansion resulted. This expansion was due to the depression caused by the high concentration of the alcohol, which was far beyond the maximum threshold of stimulation. When the fish which had their melanophores contracted in a 2 per cent solution were placed in a 0.5 per cent solution they expanded. Here the dilution of the alcohol was below the threshold stimulus. If embryos that were exposed to $7\frac{1}{2}$ per cent solution for an interval of four to six minutes, were placed in water or very weak alcohol, there was observed a contraction of the pigment cells which was of a very short duration. This result was no doubt due to the washing out or the dilution of the alcohol within

the tissues, to the threshold stimulus and as the process of dilution continued the point was reached where the concentration fell below the threshold and a relaxation (expansion) of the melanophores occurred. After a complete recovery of the embryos from the effects of the alcohol the pigment cells reacted normally to other stimuli.

These results show clearly that very weak solutions of ethyl alcohol do not have any effect on the pigment cells of the trout embryos. This is in harmony with the work of Kobert ('82) on the frog's muscle, Lee and Salant ('02) on the gastrocnemius muscle of the frog, and Carlson ('06) for the heart muscle and heart ganglion of Limulus, all of whom observed that weak or very weak solutions of ethyl alcohol had no stimulatory action. Ethyl alcohol in contractions of 1.3 per cent to 2.5 per cent water shows a decided stimulatory action on the pigment cells of brook trout embryos. This is in accord with results of others on the primary stimulation of ethyl alcohol. Pickering ('95) has shown that alcohol excites the embryonic heart muscle of the chick. Scheffer ('00) has observed that in the frog's gastrocnemius when it was treated with alcohol the capacity for work was increased. If the muscle was curanized the stimulating effect of alcohol was nil. O. Loeb ('05) has noted that in solutions of 0.13 to 0.3 per cent that the action of the isolated mammalian (cat) heart was augmented. Wood and Hoyt ('05) have shown that small amounts of ethyl alcohol increased the force of the heart beat in the frog, snake, tortoise, and turtle. Lee and Salant ('02) have demonstrated that in medium concentrations of ethyl alcohol there was an increased rate of contraction and relaxation in frog's muscle (gastrocnemius). Carlson ('06) has observed that for the heart muscle and heart ganglion of Limulus, alcohol stimulated. Vernon ('10) has shown that alcohol has an excitatory effect on the isolated heart of the turtle (Emys). The ('02) observed a marked increase in the number of contractions of the bell of the Medusa Gonionema in ethyl alcohol of 0.5 to 0.25 per cent.

In a strong concentration of 4.5 per cent there was a marked depression or an expansion of the pigment cells. In this con-

centration there was no primary stimulating period observed. If it is to be found, it may be so short as to be very easily overlooked. Alcohol in large amounts decreased the rate of contraction in the gastrocnemius frog's muscle, Lee and Salant ('02). Romanes ('77) found that strong solutions of ethyl alcohol produced increased and spasmodic contraction of the medusa bells of Sarsia (sp.) and Tiaropsis (sp.). These were followed by a depression.

Lee ('02) observed that in solutions of ethyl alcohol of a greater concentration than 2 per cent the contractions of the bell of the medusa, Gonionema, were much reduced in volume and in number. Dogiel ('77) has shown a depression in the heart rhythm of Corethra plumicornis. Vernon ('10) observed that large doses of ethyl alcohol depressed the rate and volume of the contraction of a turtle's heart (Emys).

3. Propyl alcohol. Weak solutions of propyl alcohol 0.01 per cent to 0.04 per cent did not effect the melanophores. In a 0.06 per cent there was a noticeable contraction of the pigment cells. Solutions of 0.08 per cent to 0.125 per cent produced a rapid and complete contraction. In 0.7 per cent to 1.3 per cent the contraction was only temporary, and was followed by an immediate relaxation of the pigment cells. A 1.5 per cent to 2 per cent produced no visible change in the expanded melanophores, and when embryos with contracted melanophores were exposed to the solution the melanophores expanded. In these concentrations there was observed a marked disintegration (cytolysis) of the cells. Higher concentrations (2.5 per cent to 4 per cent) killed the embryos without inducing any change in the expanded pigment cells. Contracted cells exposed to these solutions expanded instantaneously and after this response gave no reactions to other stimuli.

It is obvious from these results that the stimulation of the pigment cells by propyl alcohol begins in solutions of lower concentrations than it does in ethyl and methyl alcohol. It will be seen that my results for methyl, ethyl, and propyl alcohols are in perfect agreement with the results on the toxicity of the above alcohols of other investigators.

Joffroy and Serveaux ('95) studied the toxicity of alcohols on mammals by intravenous injections. Bear ('98) introduced the alcohol directly into the stomach of the mammals. Picaud ('97) placed fish and amphibians in the solutions of the alcohols and in this way determined the toxicity of the alcohols. Bradbury ('99) and Cololian ('01) used fish, Overton ('01) on tadpoles employed the same method in their investigations. Wirgin ('04) determined the concentrations at which the various alcohols inhibited the growth of Micrococcus pyogenes aureus. He also investigated the laking power of the alcohols on the red corpuscles of the rabbit. Vernon ('11) studied the depression of an isolated tortoise heart by the alcohols. In table 3 the toxicity of ethyl alcohol is taken as unity and the values given are the comparative toxicities of the other alcohols. The values are only approximate.

TABLE 3

ALCOHOL	JOFFROY, MAMMALS	BAER, MAMMALS	PICAUD, FISH	BRADBURY, FISH	COLLOIAN, FISH	OVERTON, TADPOLES	WIRGIN, BACTERIA	WIRGIN, RED CORPUSCLES	VERNON, TORTOISE HEART	STIMULATION OF THE PIGMENT CELLS OF TROUT EMBRYOS	DEPRESSION OF THE PIGMENT CELLS OF TROUT EMBRYOS
Methyl	0.46	0.8	0.67	1.0	1.1	0.73	0.73	0.84	0.72	0.45	0.55
Ethyl	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Propyl	3.5	2.0	2.0	1.0	3.6	2.0	1.5	2.1	2.1	2.0	3.0

The stimulation level is lowest in methyl alcohol (4.5 per cent); next is ethyl alcohol (1.6 per cent to 2.5 per cent); and lastly propyl alcohol (0.08 per cent to 0.125 per cent). This is in harmony with the results of other investigators on the toxicity of alcohols where it was found that methyl was less potent in bringing about narcosis, and the potency increased for the other alcohols directly with the molecular weight. It was shown by Baer ('98) that the toxicity of the alcohols varied directly as their boiling points. Meyer ('99) and Overton ('01) discovered that the narcotic action of the alcohols varied with their solvent power for fats or lipoids. It may be suggested that in addition to the above physical factors involved in the action of the alcohols, that the dielectric constant of the alcohols probably plays

an important part in their action. It was observed that the greater the dielectric constant of the alcohols used the lower the stimulating or depressing power, and conversely the lower the dielectric constant the more striking were the reactions. Whatever may be the relation of these physical factors of the alcohols in stimulation or depression, their chemical structure must not be overlooked; for as the length and complexity of the chain in monohydric alcohols increases so does the strength of their action.

ALCOHOLS	MOLECULAR WEIGHT	DIELECTRIC CONSTANT AT 20°C	BOILING POINT, °C.	STIMULATING POWER—ETHYL ALCOHOL TAKEN AS 1
Methyl	32.03	31.2	65.7	0.45
Ethyl	46.05	25.8	78.4	1.0
Propyl	60.06	22.0	97.4	2.0

Reactions to alkaloids

The study of the action of drugs on the pigment cells of trout was undertaken with a threefold purpose, viz., to compare the action of drugs on the pigment cells with that of other tissues; second, to determine if possible the controlling mechanism of the pigment cells, and third, to see if the drugs had a specific action on the pigment cells.

The literature on the pharmacology of the pigment cells of fish is not very extensive. The earliest historical record of experiments on the action of drugs on the pigment cells is that of Redi (1664), who observed that eels which died in a tobacco decoction were light in color. Pouchet ('76) observed that Gobius niger changed in color when placed in strychnine. Morphine, quinine, and santonin had no effect. Lode ('90) concluded that curare destroyed the nerve endings of the pigments cells of trout (Salmo fario). von Frisch ('11) found that chloral hydrate contracted the pigment cells of the minnow and crucion. He also concluded that the action of cocaine was through the central nervous system.

The pigment cell may be stimulated or depressed by the drug acting: 1) on the pigment cell in such a way as to increase or

decrease its irritability; 2) on the nerve endings leading from the ganglia controlling the pigment cells; 3) on the central nervous system. I have no direct evidence to offer which will enable us to determine which of these or combination of these three factors are operative in the action of the drugs on the pigment cell, for I was unable to separate the nervous and pigment cell tissues for experimental purposes. It is obvious that large doses have no selective action. At certain optimal concentrations all the drugs show a selective action on the pigment cells or their controlling mechanism. This selective action of drugs on the mechanisms of the pigment cells will further our knowledge as to their function.

Fig. 1 A normal brook trout embryo showing the general alignment of the body.

In interpreting my results I have given special emphasis to their relation in a comparative way to the observations of other observers on various vertebrate and invertebrate tissues. This comparative method makes the results easier of interpretation and is less liable to lead to an erroneous conclusion.

The drugs used were all of Merck's manufacture. They were dissolved in oxygenated distilled water. The stock solutions were made up from 0.25 per cent to 0.5 per cent. Dilutions were made from these solutions. The experiments were carried on in Syracuse watch glasses in 10 cc. of the solution. These results were checked by experiments in small stender dishes of 50 cc. capacity. The conclusions are based on experiments repeated ten times in 1913 and again in 1914 another series of ten was tried. Five to ten animals were used at one time in each dilution. The trout embryos used were from four days to two weeks after hatching. In no case were the individuals of the different ages mixed.

[2] Figures 1, 2, and 3 were drawn by Miss H. J. Wakeman.

Other experiments are in progress to determine the action of drugs on pigment cells isolated from the nervous system.

1. Strychnine. In 0.5 per cent oxygenated solution death resulted without primary stimulation of the pigment cells. In 0.05 per cent strychnine solution the results were the same. Solutions of 0.005 per cent strychnine caused a contraction of the pigment cells rapidly, the contraction was complete in five minutes. There was a remarkable thing observed in this concentration of strychnine. The irritability of the fish was increased to a high degree. The fish went into typical strychnine spasms. The head was thrown backward and the tail curved upward and forward, describing a half circle (as shown in text fig. 2). A

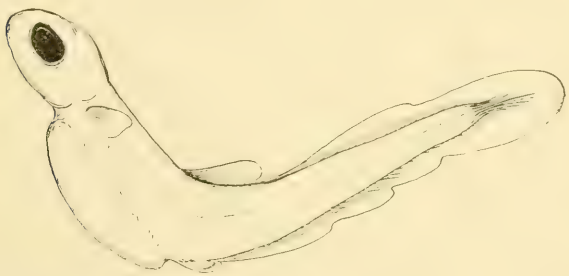

Fig. 2 Showing a brook trout embryo in a typical opisthotonos response in 0.005 per cent strychnine.

passing shadow over the disk brought on a new spasm. Shadows in rapid succession increased the concavity backward. If the dish was tapped very lightly the same responses occurred. This period of heightened excitability lasted from eight to twelve minutes. During this interval the pigment cells remained contracted (fig. 13). As this convulsive period disappeared, the pigment cells expanded (fig. 14). This expansion showed that the depression and paralysis of the pigment cell controlling mechanism had occurred. In 0.0005 per cent the pigment cells were contracted in ten minutes. No convulsions were observed in this concentration. In weak solutions of 0.00005 per cent to 0.000025 per cent no contractions of the melanophores was produced.

Pouchet ('76) observed that the pigment cells of Gobius niger contracted in strychnine solutions. Romanes ('77) noted that in the medusa Sarsia (sp.) the swimming motions were much accelerated by strychnine, also that convulsions occurred in this and three other forms—Cyanaea capillata, Tiaropsis indicans, and Tiaropsis diademta. Hedborn ('99) has shown that strong doses of strychnine augment the beat of the isolated mammalian heart (cat). Dogiel ('77) demonstrated a slight increase in the rate of the heart beat of Corethra larvae. Pickering ('93) observed that weak solutions of strychnine had a primary stimulating action on the heart muscle of an embryonic chick. Carlson ('06) has found that strychnine in very weak concentrations had a distinct stimulatory action on the heart ganglion of the Limulus heart. Stronger solutions produced augmentation followed by paralysis. He was unable to note any primary stimulation on heart muscle. Laurens ('15) observed that if a drop or two of a 1 per cent solution of strychnine was injected into the body cavity of Amblystoma larvae the pigment cells contracted.

All the above experiments on other tissues show that strychnine has a primary stimulating action and especially on the motor ganglia. From the evidence of Ballowitz ('93) who demonstrated that the pigment cells of fish have a connection with the nervous system, and from the fact that strychnine stimulates the nervous system, we are warranted in concluding that strychnine acts directly on the nervous mechanism controlling the melanophores of trout embryos, rather than on the melanophores themselves. The seat of strychnine poisoning is in the spinal cord, therefore, the melanophores of trout embryos are in all probability controlled in part by the spinal nervous system.

2. Picrotoxin. Picrotoxin is used as a fish poison. It produces a medullary stimulation and ultimately results in death. When trout embryos are exposed to a 0.25 per cent solution of picrotoxin the pigment cells contract rapidly. The contraction is complete in two to five minutes. The contraction remains for forty-eight to sixty-four hours, if the fish are kept in this solution. The fish live in 0.25 per cent solution for one hundred

and twenty-six hours. There is no convulsive period. In a weak solution of 0.025 per cent of pictoroxin the contraction is slightly less rapid, and lasts indefinitely (fig. 11).

When the tail is cut away the pigment cells in the tail portion expand (fig. 12). They remain expanded for six hours and then degeneration sets in. The melanophores in the anterior or head end remain contracted. The contraction continues for eight to twelve hours and then disintegration of the pigment cells occurs. This justifies the conclusion that the reactions of the pigment cells of trout embryos are in some way controlled by the higher nerve centers.

If the pigment cells that are contracted in picrotoxin are expanded in 0.2 M. NaCl and are now placed in picrotoxin the contraction is much slower than the first time. The sodium chloride seems to counteract the action of the picrotoxin.

3. Morphine. In embryos exposed to 0.5 per cent solution of morphine hydrochloride the pigment cells remain expanded. In a 0.12 per cent most of the pigment cells were expanded but there were a few isolated areas that showed a contraction. After an exposure of three hours these isolated areas of contracted pigment cells had increased. In a 0.06 per cent solution of morphine the result was the same. In a 0.012 per cent solution no change occurred, all the pigment cells remained expanded. There was no contraction of the pigment cells in a 0.005 per cent solution. Pigment cells contracted by picrotoxin, potassium iodide or strychnine were expanded by morphine. According to Pouchet ('76), morphine did not cause any change in the pigment cells of Gobius niger. Romanes ('77) has found that in Aurelia aurita morphine had a highly depressing action. Pickering ('93) found that morphine acetate depressed the action of the heart muscle of embryo chicks. Cushny ('10) says that the action of morphine on the central nervous system is a mixture of stimulation and depression which are not equally marked throughout the system; also, "there is a selective action on the medulla oblongata in which certain centers are entirely paralyzed before neighboring ones undergo any distinct modification." Waller ('96) found that morphine applied directly to the nerve had but little effect on its irritability.

The explanation for the localized areas of contracted pigment cells may depend upon the selective action of morphine upon the nervous system.

The foregoing experiments support the conclusion that the pigment cells are controlled by the medulla or the spinal cord. It is probable that the localized areas of expanded and contracted pigment cells are in direct response to the mixture of stimulations and depressions caused by the action of morphine on the medulla. Or if the pigment cells are controlled by the reflex irritability of the spinal cord which may be depressed for a period and then may be followed by an increased irritability. On the latter hypothesis all the pigment cells should contract during the heightened irritability or expand during the diminished irritability; but since this is not the case it is probable that all the regions of the spinal cord are not involved at the same time.

4. Caffeine. In embryos exposed to a 0.2 per cent to 0.25 per cent solution of caffeine citrate no change occurred in the pigment cells. The animals died in a much distorted condition. The pigment cells disintegrated in two hours. A 0.05 per cent solution of caffeine citrate caused the pigment cells to contract in 4.25 minutes. There was a peculiar twitching of the muscles which lasted twelve minutes. A depression occurred in fourteen minutes. The pigment cells expanded very rapidly. In 0.025 per cent caffeine citrate solution contraction of the pigment cells took place in 5.25 minutes. The depression or paralysis was elicited in thirty minutes in some, while in others it took forty-five to sixty minutes. A solution of 0.005 per cent caffeine citrate caused no contraction of the pigment cells in two and one-half hours.

The convulsions observed were quite similar to those that occurred in strychnine. In caffeine the responses to shadows were absent. If the dish was jarred the reactions were weaker and lasted a short interval. These reactions occurred in solutions ten times as strong as in strychnine. The response was not opisthotonus, but the head was drawn toward one side and the tail toward the other. There was no difference in the sides to which the curvature occurred (as shown in text fig. 3). The

animal was in the form of the letter S. The convulsive period lasted a short time and gave from one to six spasmodic reactions. The pigment cells remained contracted during this period. As the convulsive tremors gave way to a complete paralysis the pigment cells expanded. The convulsive period and the contraction of the pigment were simultaneous. Weak solutions of 0.0005 per cent had no effect on the trout embryos or their pigment cells.

If the embryos are removed from a 0.05 per cent caffeine citrate solution during the period of convulsions, and if the poison is washed out rapidly there is a complete recovery. The pigment cells expand normally. If removed during paralysis after con-

Fig. 3 Showing a brook trout embryo in a typical caffeine convulsion.

vulsions the fish may recover very slowly or not at all. In weaker solutions of 0.01 per cent to 0.025 per cent there are no convulsions, but only a contraction of the pigment cells; there is a complete recovery when they are placed in water.

Carlson ('06) has shown that caffeine caused a primary augmentation in the heart muscle and primary stimulation of the heart ganglion of Limulus. Hedborn ('99) observed that caffeine stimulated the isolated mammalian heart (cat). Pickering ('93) observed an increase in the number of heart beats in the embryo chick's heart, and concluded from his work that it is not necessary to introduce a nervous hypothesis to explain the action of caffeine. Romanes ('77) has found in Sarsia (sp.) exposed to a sea water saturated with caffeine there was a great increase of the contraction and at the same time a diminution

of the potency of the beat. Soon the pulsations became of a fluttering nature and spontaneous movements ceased.

In the pigment cells of trout there is a stimulation which is at its height during the convulsive period. This is soon followed by a paralysis and an expansion of the pigment cells results. There is a direct resemblance in the results obtained with caffeine and strychnine, in that the reflex irritability is remarkably increased. The pigment cells contract in both instances during the convulsive period. There is a similarity in the results on the pigment cells of trout and the work of other investigators on other tissues. Caffeine may act directly on the pigment cells as it does on muscle (Pickering, '93, Carlson, '06), or it may stimulate the reflex centers in the medulla and spinal cord, which give off the fibers which control the pigment cells.

5. Curara. In very strong solutions of curara of 2 per cent to 1 per cent, a few pigment cells contracted. When trout embryos were exposed to a 0.5 per cent solution they moved about rapidly for eight to ten minutes. In one case one showed a complete contraction of the melanophores in three minutes while the other nine fish in the same lot showed no change. The one that showed this contraction had its pigment cells completely expanded in thirteen minutes. In 0.25 per cent solution there occurred a partial contraction of the pigment cells. The pigment cells along the lateral line were not contracted. The fish died in an hour and were covered with a colorless jelly-like slime. In the following dilutions of curara, viz., 0.05 per cent, 0.025 per cent and 0.001 per cent a partial contraction of the pigment cells occurred in two minutes and thirty seconds. In a 0.0025 per cent curara solution the change took place in fourteen to forty minutes. In all the experiments the contraction of the pigment cells was not evenly distributed but occurred in spots (fig. 15). The tail portion showed many contracted pigment cells, but in the head region there were the largest number of contracted melanophores. Along the lateral line the pigment cells remained expanded. After fourteen or fifteen minutes all the contracted pigment cells were expanded. This mixture of responses was constant for all the experiments.

Pouchet ('76) observed that curara did not modify the reaction of the pigment cells of turbot, viz., the pigment cells remained in an expanded condition. Lode ('90) has found that subcutaneous injection of a mixture of curara and glycerine caused a dark coloration in the adult trout (Salmo fario). He ligated the aorta and found that in the anterior end with the intact circulation expansion of the pigment cells occurred, while in the posterior end with the interrupted circulation, the pigment cells remained contracted. These experiments are not conclusive because the removal of the circulation interfered with normal metabolism of the cells. Moreover, the pigment cells are very sensitive to the changes in their oxygen supply. He observed that if the spinal cord of a curarized trout was stimulated, no contraction of the pigment cells occurred. If the pigment cells were stimulated directly, the pigment cells contracted. He concluded that the curara destroyed the nerve endings but did not affect the pigment cells. Laurens ('15) found that if Amblystoma larvae were placed in a 0.2 per cent solution of curara their movements were abolished and the pigment cells remained expanded under all conditions. He concluded that this failure on the part of the pigment cells to react was probably due to the direct effect of the solution on the animal; or asphyxiation of the larvae by the curara, and the consequent increased amount of CO_2 in the blood may have caused the melanophores to remain expanded. If a small amount of 1 per cent solution of curara was injected into the body cavity the larvae were rendered unmotile but the melanophores reacted to light (expanded) and to darkness (contracted) as usual. He concluded here that this experiment did not prove that curara had no effect on the melanophores, for it has been shown that melanophores will contract and expand after all nervous connections have been destroyed.

Carlson ('06) has shown that in weak solutions of curara there was a primary stimulation of the heart ganglion of Limulus. It had a little effect on the heart muscle. Young ('81) observed that in Mya (sp.) and Solen (sp.) there was a distinct acceleration in the number of heart beats, and sometimes a diminution,

and even a complete arrest. Plateau ('80) found that curara did not modify the frequency of the amplitude of the Decapod heart. Larger doses diminished the amplitude. Dogiel ('77) showed there was a primary stimulation by curara of the heart of Corethra plumicornis. Boehm and Tillie ('04) have observed a primary stimulation of the isolated mammalian heart (dog).

Since Curara stimulates the central nervous system Cushny ('10) and other ganglia Carlson ('06), it is possible that it acts as a stimulant on the medulla and spinal cord which transmit the impulse to the chromatophores, and a contraction results. Later as the curara destroys the nerve end plates the stimulus does not reach the pigment cell from the center. The pigment cells retain their independent irritability for a long time. The mixture of contracted and expanded melanophores is probably due to the unequal action of the curara on the peripheral nervous mechanism of the melanophores.

6. Nicotine. When trout embryos were exposed to 0.5 per cent nicotine solution, their muscles twitched for a moment and then all activity ceased. The heart beat continued for twenty-eight minutes. There was no change in the pigment cells. The pigment cells disintegrated soon after death. There was a very marked maceration of all the tissues. The whole fish was covered by a colorless slime. In a 0.125 per cent nicotine solution there was a slight primary contraction of the melanophores which was followed almost simultaneously by an expansion. The eyes bulged out of the head which caused the fish to appear grotesque. A 0.005 per cent solution of nicotine caused a complete contraction of the pigment cells in two and one-half minutes. The paralytic expansion occurred eight minutes after the contraction. In 0.0025 per cent nicotine the contraction time was the same as in the preceding experiment. The period of paralysis was delayed which occurred in eleven minutes. A nicotine solution of 0.0005 per cent produced a complete contraction in eleven minutes. The paralysis or depression of the pigment cells appeared in thirty-five minutes. In a 0.0001 per cent nicotine solution there occurred only a very slight change in the form of the pigment cells. In diluted 0.00005 per cent

no change was elicited. In all the cases where paralytic expansion occurred, it appeared first on the ventral side. The reason for this is not understood.

Redi (1634) according to van Rynberk ('05) observed that eels which died in a tobacco solution became lighter in color. Cushny ('10) says, that in nicotine the spinal cord is thrown into a condition of exaggerated irritability and that the medulla seems to be involved to a greater degree than the spinal cord. The stimulation does not involve the higher brain centers. Carlson ('06) observed that nicotine in weak solutions stimulated the heart ganglion of the Limulus heart. This primary stimulation was followed by a depression. There was no primary stimulation of the heart muscle. Gee ('13) has found that in a solution of 0.00066 per cent of nicotine leeches were vigorously stimulated, which was followed by a depression of movements. Romanes ('77) found that violent spasms were incited in the medusae Sarsia (sp.) and Tiaropsis when exposed to nicotine. He also observed various distortions. Langley and Dickson ('90) concluded that nicotine acts directly on the nerve cells and not on the muscle.

It is known that nicotine first stimulates and later paralyzes the ganglionic cells of the sympathetic system, whether applied directly to them or injected into the circulation. It is quite probable that nicotine affects the sympathetic system of the pigment cells, for there is first a contraction of the cells which is later followed by an expansion.

7. *Atropine.* Strong solutions (0.5 per cent) produced no change in the pigment cells. The fish lived four hours in this concentration. In 0.025 per cent solution of atropine sulphate, there was no change in the pigment cells. All possible concentrations were tried, but none of them produced a contraction of the pigment cells.

Pigment cells were contracted in 0.005 per cent strychnine solution and then were transferred to solutions of 0.05 per cent to 0.0025 per cent of atropine, where all the pigment cells expanded rapidly. The expansion was complete from two to four minutes. Pigment cells contracted in potassium salts were expanded just as those that were contracted by strychnine.

All the experiments show conclusively that atropine does not have any direct stimulating action on the pigment cells of trout embryos. Cushny ('10) says, that atropine acts on the higher centers of the brain and less on the lower divisions, viz., the medulla and the spinal cord, which is just the reverse of strychnine. This acts on the lower centers and not on the central system. The results obtained justify the conclusion that the pigment cells are controlled by the lower reflex centers.

Romanes ('77) in his experiments on the medusae Sarsia (sp.) and Tiaropsis found that atropine caused convulsive swimming movements by a marked depression. Pickering ('93) showed that 0.012 gm. of atropine to 1 cc. of normal saline solution reduced the normal heart beat of the embryonic heart of the chick. Carlson ('06) found that atropine stimulated the heart ganglion and not the muscle of the Limulus heart. Cushny ('10) says most secretions are depressed by the administration of atropine. This is not due to the inactivation of the secretory cells, but to the failure of the nervous impulses. The action of atropine on other tissues, from all evidence, shows us that it does not act directly on the vascular and secretory elements, but on their nerve terminations. It is therefore possible that atropine acts on the pigment cells through their nerve fibers, paralyzing them, but does not act directly on the pigment cells.

8. Cocaine. One-half per cent solutions killed the trout embryos rapidly. There was a momentary stimulation of the pigment cells which was followed almost simultaneously by an expansion of the pigment cells. In a 0.125 per cent solution of cocaine the behavior of the pigment cells was the same as in the 0.5 per cent solution. In 0.025 per cent to 0.05 per cent cocaine solution the pigment cells were contracted in four minutes. The contraction was followed by an expansion. Solutions of 0.005 per cent of cocaine produced a complete contraction in five minutes. The expansion followed in twelve minutes. Very weak, 0.00033 per cent solutions, had no effect on the melanophores.

These results show that cocaine has a primary stimulating action on the pigment cells of trout embryos. This primary

stimulation is followed by an expansion of the pigment cells. The action of cocaine on the nervous system is in a series, namely, the cerebrum is first affected, then the cerebellum and medulla, and lastly the spinal cord. It also acts on the sensory fibers and their terminations.

Von Frisch ('11) observed that the local application of cocaine caused the contraction of the melanophore in the minnow and Carssius (sp.). An injection of a 5 per cent solution into the body cavity caused a contraction of the pigment cells after the sympathetic nerves were severed. He concluded that the action of cocaine was through the central nervous system. Carlson ('06) showed that weak solutions of cocaine had a primary stimulating action on the heart ganglion of Limulus, but had no effect on the heart muscle. Hedborn ('99) observed a slight primary stimulating action on the isolated heart of the cat.

It is probable that cocaine acts on the reflex center which controls the pigment cells. It may act on the nerve endings of the pigment cells. It is obvious that it will require a great deal more of work to determine the relation of cocaine to the pigment cells before any generalization can be made.

9. Veratrine. Solutions of veratrine of 0.5 per cent concentration caused a rapid contraction of the pigment cells. The contraction was complete in two minutes. Paralysis set in at six minutes, and pigment cells were completely expanded in two more minutes. In a 0.25 per cent solution the stages were the same. In a 0.005 per cent veratrine solution the pigment cells were completely contracted in nine minutes. The first signs of paralysis appeared in eighteen minutes. Veratrine was a very active agent in causing the contraction of the pigment cells. Dilutions of 0.0005 per cent to 0.00005 per cent caused a contraction of the pigment cells in eighteen to twenty minutes. The paralytic expansion occurred in thirty minutes. A solution of 0.00001 per cent veratrine caused no change in the pigment cells.

Veratrine acts on the medullary center and the spinal cord, where a marked increase of irritability is elicited. After large doses there is a paralysis of the centers. It acts on the periph-

eral ganglia and nerve endings. It is highly probable that the action of veratrine on the pigment cells is through the lower centers of the nervous system rather than local.

Carlson ('06) found that weak solutions of veratrine had a primary stimulating action on the heart ganglion of Limulus. In strong solutions the period of stimulation was followed by a depression in two minutes. The ganglion free heart did not respond to the poison. Plateau ('78) observed a primary stimulation in the heart of Carcinus moenas and Homarus (sp.) which was followed by a depression. Romanes ('77) found that in the medusa Sarsia (sp.) the first effect of veratrine was an increase in the number and potency of the contractions. This period of increased responsiveness was followed by a gradual depression into complete quiescence.

Summarizing the action of veratrine on the pigment cells, it may be stated, that it first stimulates the contraction of the pigment cells. This period of stimulation is followed later by a paralysis of the mechanism controlling the pigment cells. The pigment cells are expanded during this period of depression. This is in harmony with the observations of other workers on various tissues, where there is observed a primary stimulation followed by a depression.

10. Quinine. Quinine in a 0.5 per cent maintained for a long time the pigment cells in an expanded condition. Dilutions were made from 0.25 per cent to 0.0005 per cent of quinine hydrochloride solution, and in all of these dilutions no change occurred in the pigment cells. Solutions of 0.000033 per cent to 0.0000165 per cent gave the same result.

The pigment cells were first contracted in picrotoxin and were then placed in the quinine solutions of 0.025 per cent to 0.005 per cent and in every case a rapid expansion of the pigment cells occurred. The rapidity of the expansion was greater in the quinine than it was in the ordinary process of washing out of the picrotoxin.

Quinine differs from most drugs in that its action is very widespread, and it is often called a general protoplasmic poison. Binz ('68) observed that quinine inhibited the beat of the cilia

in protozoans. Also, that it stopped the movements of the leucocytes. Santesson ('93) found that quinine depressed the rhythm of an isolated frog's heart. Hedborn ('99) observed that quinine depressed an isolated mammalian heart (cat). O. and R. Hertwig ('87) observed that sperm treated with quinine had their movement paralyzed. Eggs when treated with quinine after the sperm entered, the conjugation of the pronuclei was delayed. Carlson ('06) has found that quinine did not stimulate the ganglion or the muscle of the Limulus heart.

It is obvious from the experiments that quinine exhibits no primary stimulating action on the pigment cells of trout embryos. Any accurate interpretation of the depressing action of quinine is not possible, since the drug acts in the same way on the nervous tissues and the pigment cells as well.

SUMMARY

1. The experiments were performed on the melanophores (pigment cells) of the brook trout embryos, Salvelinus fontinalis Mitchill. Such young trout have only one kind of pigment cells, the melanophores. The young two-day or two-week old trout do not yet react to back ground. The first sign of reaction to back ground appears only after the yolk is absorbed.

2. In the presence of oxygen the pigment cells remain expanded and the fish live indefinitely. When hydrogen (oxygen want) is substituted for the oxygen the pigment cells contract and the embryos die. Oxygen is necessary for the maintenance of the expansion of the melanophores and life of the trout embryos.

3. Carbon dioxide excess caused a contraction of the melanophores. If oxygen was bubbled with the carbon dioxide, the presence of the oxygen had an antagonistic action.

4. Distilled water caused a rapid contraction. A mixture of distilled and boiled tap water gave the same result. In boiled tap water the pigment cells contracted. Oxygenated distilled water and boiled tap water maintained the pigment cells in a normal expanded condition. It was the absence of oxygen and not of the salts that caused the contraction.

5. In the potassium salts, K_2SO_4, KCl, KBr, KNO_3, and KI there occurred a rapid contraction of the expanded melanophores. The rate and degree of the contraction was the order given

$$I > \quad NO_3 > \quad Br > \quad Cl > \quad SO_4$$

This primary contraction was followed by a cytolytic degeneration (expansion). The time required for the appearance of this degeneration was greatest in

$$I > \quad NO_3 > \quad Br > \quad Cl > \quad SO_4$$

If the contraction or degeneration of the melanophores is specific for the potassium cation, it is unqualifiedly modified by its anion, or the residual part of the undissociated molecules.

6. The neutral salts of sodium, Na_2SO_4, NaCl, NaBr, $NaNO_3$, and NaI, caused a slow contraction of the melanophores. The contraction was most rapid in NaI and slowest in Na_2SO_4 and other salts were intermediate as

$$I > \quad NO_3 > \quad Br > \quad Cl > \quad SO_4$$

Degeneration appeared first in NaI and last in Na_2SO_4 and varied in this order

$$I > \quad NO_3 > \quad Br > \quad Cl > \quad SO_4$$

The irritability of the chromatophores and life of the fish was maintained longest in Na_2SO_4 and NaCl from (118 to 132 hours) in NaI from one to two and one-half hours.

7. The pigment cells that were contracted in potassium salts, when placed in sodium salt they expanded. The order of expansion was

$$SO_4 > \quad Cl > \quad Br > \quad NO_3 > \quad I$$

There was no expansion in NaI.

8. The results obtained in the experiments on the action of the salts on the pigment cells of trout are probable to be explained on one or more of three assumptions: (1) That it is due to the antagonistic action between anion and cation; (2) that it is the independent action of the cation; (3) that reaction of the melanophores is likely modified by the undissociated molecule.

9. The narcosis or depression of the pigment cells of trout by the homologous alcohols corresponds very closely to their narcotic action as determined by Overton and numerous other investigators.

10. Very dilute solutions of methyl, ethyl, and propyl alcohols exert no action on the pigment cells of trout.

11. The pigment cells of trout embryos respond to alcoholic stimuli. Their mode of reaction is comparable to the reaction of other tissues to alcohols inasmuch as they are stimulated by small doses and depressed by large doses.

12. Strychnine in moderate doses causes a primary contraction of the expanded melanophores. Large doses cause a depression without a primary stimulation (contraction). The action of the strychnine is on the nervous system rather than on the pigment cells directly.

13. The action of picrotoxin causes a rapid contraction of the pigment cells. The mechanism controlling the pigment cells is in the higher centers, because if the spinal cord is severed the pigment cells expanded.

14. Morphine induces a contraction of the melanophores in isolated areas. This is probably due to the selective action of morphine upon the nervous system. Large doses produce no change in the expanded melanophores. Morphine expands the pigment cells that were contracted in picrotoxin, KCl, and strychnine.

15. Curara causes a mixture of responses, that is, there are areas of expanded and contracted melanophores. This is likely due to the unequal action of the curara on the peripheral nervous mechanism of the melanophores.

16. Medium solutions of nicotine cause a contraction of the pigment cells. Strong nicotine solutions have no effect on the pigment cells. The action of nicotine is directly on the nervous controlling mechanism of the pigment cells.

17. Atropine in all concentrations has no stimulating action on the pigment cells of trout. Atropine paralyzes the fine nerve connections of the pigment cells.

18. Cocaine has a primary stimulating action on the pigment cells of trout. This action is probably on the nerve endings of the pigment cells that connect them with the reflex center.

19. Veratrine causes a primary contraction of the pigment cells which is followed by a rapid depression (expansion). The action of veratrine is through the reflex center of the spinal cord and medulla rather than local.

20. Quinine exhibits no primary stimulating action on the pigment cells. The drug has no selective action on tissues, therefore it is a general 'protoplasmic poison.'

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PLATE 1

EXPLANATION OF FIGURES

In figures 1 to 10 are shown five sets of brook trout embryos which were exposed to the action to 0.2 M solutions of Kl, KNO_3, KBr, KCl, and K_2SO_4, 1 to 5 for an interval of fifteen minutes, and figures 6 to 10 for a period of three hours.

1 The melanophores were completely contracted in Kl.

2 The contraction was not as pronounced in KNO_3.

3 In KBr the melanophores had longer processes than in the two preceding solutions.

4 In KCl the processes were more distinct and showed the finer arborizations.

5 An exposure of fifteen minutes to K_2SO_4 produced no observable change in the melanophores.

6. After an exposure of three hours to Kl the melanophores showed a distinct secondary expansion.

7 In KNO_3 an exposure of three hours produced a less extensive secondary expansion than Kl.

8 In KBr the processes were very much shorter than in Kl and KNO_3.

9 After three hours in KCl the melanophores were still spherical, but there was a suggestion toward a peripheral migration of the pigment as indicated by the swollen condition of the cells.

10 After three hours in K_2SO_4 there was no expansion of the melanophores.

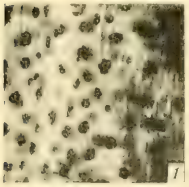
11 All melanophores contracted, photograph taken after twenty-four hours of exposure to Picrotoxin.

12 The melanophores expanded after severing the tail in an individual which was exposed to Picrotoxin for twenty-four hours. Photograph taken five minutes after cutting.

13 All melanophores contracted during the period of Strychnine convulsions.

14 Showing the expansion of the melanophores after the strychnine convulsion had subsided.

15 The contracted and expanded melanophores as they occurred in 0.0025 per cent curare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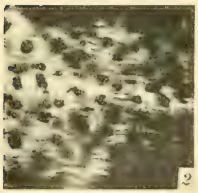
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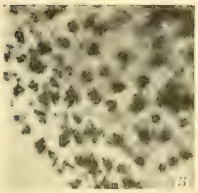
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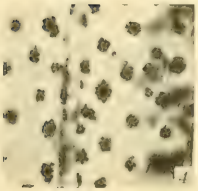
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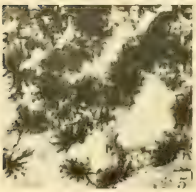
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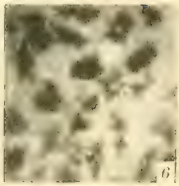
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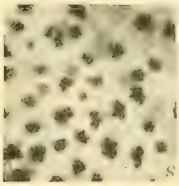
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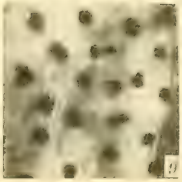
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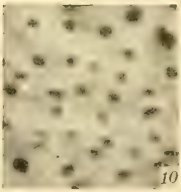
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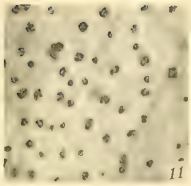
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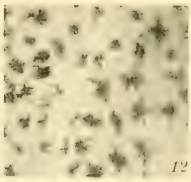
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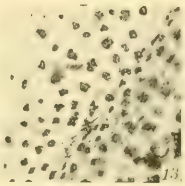
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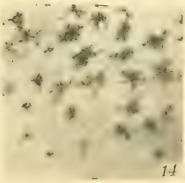
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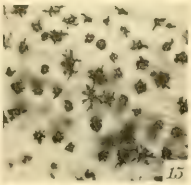
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